

Original Article

Tracing the success of scaling and root planning (SRP) in patients with chronic periodontitis by salivary nitric oxide

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Abstract

Introduction: Salivary biomarkers may elucidate orodental inflammatory processes. Nitric oxide (NO) may help us to diagnose such changes.

Methods: In this case-control study, all referral patients diagnosed with 1<clinical attachment loss (CAL) ≤4mm in >30% sites were enrolled as generalized mild to moderate periodontitis group (PG). All PG and healthy control group (CG)' individuals underwent scaling and root planning (SRP). The periodontal indices were recorded at baseline (day0) in both PG and CG, in addition to 14 days after SRP (day 14) : salivary nitric oxide level on same occasions also recorded.

Results: Twenty seven individuals were enrolled as PG and 17 individuals were assessed in CG. All indices were improved with SRP after 14 days. Except for NO, none of the periodontal indices subsided to the normal values of CG.

Conclusions: Nitric oxide is a sensitive biomarker in tracing periodontal inflammation.

Keywords: Nitric oxide, Inflammation, Saliva, Periodontitis.

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Introduction

Currently, saliva received high attention as a sole or adjunct diagnostic tool in the field of dentistry (1). Nitric oxide (NO) is available by either entero-circulatory-salivary circulation or NO synthase (NOS) in mammals. The latter consists of three main isoforms; e-NOS (NOS3), n-NOS (NOS1) and i-NOS (NOS2).

Nitric Oxide Synthase 1,3 are constitutional and calcium-enzymatic dependent forms that produce lesser amount that last shorter when compared to i-NOS as an inducible form which its production is dependent on pro-inflammatory cytokines (e.g., IL₁,

TNF_α, and IFN_γ) with a destructive characteristic that is produced in a higher amount and greater durability (2). Nitric oxide participates in immune modulation (e.g., respiratory burst, neutrophil oxidative stress), decreases platelet aggregation, induces vasodilation and possesses anticariogenic, antibacterial and bone remodelling properties. The main sources of saliva may be addressed to nerve endings, secretory cells of salivary glands, salivary gland endothelial cells and oral bacteria products besides ingested nitrate and nitrites (3-5). We aimed to assess how salivary nitric oxide level reflects the success of scaling and root

planning (SRP) in patients with mild to moderate generalized chronic periodontitis.

Methods

An analytic cross-sectional study was designed and all referral patients diagnosed with clinical attachment loss (CAL) ≤ 4 mm and more than 1 mm in $>30\%$ sites were enrolled as generalized mild to moderate periodontitis group (PG).

The exclusion criteria were active oral lesions, oral cancers, salivary glands inflammation and infection, concurrent anti-inflammatory or antibiotic consumptions, cigarette smoking, prior SRP during last 6 months and bleeding diathesis. For comparison, healthy individuals with <1 mm CAL were included as CG. All PG patients were candidate for non-surgical phase I periodontal therapy including oral hygiene instructions and SRP. Periodontal indices consisting of plaque index (PI), gingival index (GI), clinical attachment loss (CAL), bleeding index (BI) and

probing pocket depth (PPD) were recorded at baseline (day₀) in both PG and CG, in addition to 14 days after SRP (day₁₄) in PG. Besides, un-stimulated saliva was obtained after 1-hour with no drinking and eating and NO content was measured with colorimetric Griess reaction at day_{0,14} using a spectrophotometer calibrated at 540 nm. Comparisons were accomplished using paired and independent t-test. The significant level was two tailed α error <0.05 . This research was approved by the Ethics Committee of Babol University of Medical Sciences.

Results

Twenty seven individuals were enrolled as PG and 17 individuals were assessed in CG. Among those, there were 10 (58.8%) females and 7 (41.2%) males in PG and 16 (59.3%) females and 11 (40.7%) males in CG. Gender distribution did not differ in groups ($p < 0.58$). The detailed descriptive and analytic data are summarized in tables 1 and 2.

Table 1. Changing Trends of Inflammatory Indices in Periodontitis Group during the Study Course

Index	Before SRP [†] (SD) ^{††}	After SRP(SD)	Effect Size(CI%95)	Significance
Probing Pocket Depth	2.84 \pm 0.41	2 \pm 0.27	0.84(0.59-1.08)	t(15)=7.33, p<0.001
Plaque Index	1.69 \pm 0.28	0.57 \pm 0.33	1.12(0.92-1.32)	t(15)=12.17, p<0.001
Gingival Index	1.79 \pm 0.24	0.86 \pm 0.1	0.93(0.82-1.04)	t(15)=18.36, p<0.001
Bleeding Index	1.69 \pm 0.33	0.9 \pm 0.13	0.72(0.57-0.82)	t(15)=10.09, p<0.001
Clinical Attachment Loss	2.91 \pm 0.37	2.33 \pm 0.35	0.57(0.47- 0.68)	t(15)=11.65, p<0.001
Nitric Oxide	169 \pm 86	79 \pm 71	90(58-122)	t(16)= 6.08, p<0.001

[†] SRP: Scaling and Root Planning ^{††} SD: Standard Deviation

Table 2. Comparison of Inflammatory Indices between control group (CG) and Periodontitis group (PG) 14 days after SRP

Index	CG [†] (SD) ^{††}	PG ^{†††} (after SRP [‡]) (SD)	pvalue
Nitric Oxide (mg)	63.47 \pm 7.84	79 \pm 71	0.4
Probing Pocket Depth (mm)	1.16 \pm 0.13	2 \pm 0.27	<0.001
Bleeding Index	0.16 \pm 0.06	0.9 \pm 0.13	<0.001
Gingival Index	0.2	0.86 \pm 0.1	<0.001
Plaque Index	0.33 \pm 0.02	0.57 \pm 0.33	<0.001

[†] CG: Control Group, ^{††} SD: Standard Deviation, ^{†††}PG: Patient Group, [‡]SRP: Scaling and Root Planning

Discussion

Based on literature, our study is among the few studies that evaluate the diagnostic role of NO for the success of phase I periodontal therapy. A significantly

lower salivary NO level was measured after SRP (effect size= 90 mg, $p < 0.001$). More recent studies, argued and agreed upon this finding as well (6, 7).

Previously, Gullu et al. showed that SRP to a higher extent modified Widman Flap (MWF) surgery attenuated inflammation measured by i-NOS expression in gingival biopsies of chronic periodontitis patients (7). This was accompanied by rising arginase level that competes with NOS for arginine as the substrate of their pathways (8). On the contrary, Gheren et al. claimed that periodontal therapy may reduce arginase level in saliva of chronic periodontitis patients (9). E-NOS and n-NOS, to a lesser extent, regulate osteoblastic function and enforce estrogen-derived bone formation (8).

Moreover, many studies clarified that i-NOS was related to gingival inflammation and bone destruction. Prostaglandins and bacterial LPS are responsible for gene up-regulation of i-NOS with subsequent osteoclastic differentiation, atherosclerosis and endothelial dysfunction in addition to down regulation of e-NOS (5).

Hence, pharmacologic suppression of i-NOS expression may clinically prevent further bone loss (e.g., Simvastatin, a lipid lowering agent, may inhibit Cyclosporine-A induced bone loss by lowering i-NOS expression (10). Newly introduced guided pocket re-colonization (GPR) may also be helpful in treating periodontal disease. GPR is an implantation of bio-friendly probiotics (e.g., *Lactobacillus Brevis*) within the depth of periodontal pocket, by which matrix metalloproteinase activity and i-NOS expression are diminished (11).

As demonstrated in the tables, all indices decreased dramatically after SRP. Nevertheless, these indices were considerably higher than the baseline indices in the CG ($p < 0.001$), except for the NO level ($p = 0.4$).

According to our findings, salivary NO may be more sensitive than clinical judgment of success of non-surgical periodontal therapy. Conclusively, salivary NO as a sensitive biomarker can also be applied when a dormant progressive inflammation in the course of periodontal diseases is suspected for the diagnostic and assessment of treatment purposes.

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